# Light-dependent subcellular translocation of $G_q\alpha$ in *Drosophila* photoreceptors is facilitated by the photoreceptor-specific myosin III NINAC

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## Summary

**We examine the light-dependent subcellular translocation of the visual $G_q\alpha$ protein between the signaling compartment, the rhabdomere and the cell body in *Drosophila* photoreceptors. We characterize the translocation of $G_q\alpha$ and provide the first evidence implicating the involvement of the photoreceptor-specific myosin III NINAC in $G_q\alpha$ transport. Translocation of $G_q\alpha$ from the rhabdomere to the cell body is rapid, taking less than 5 minutes. Higher light intensities increased the quantity of $G_q\alpha$ translocated out of the rhabdomeres from 20% to 75%, consistent with a mechanism for light adaptation. We demonstrate that translocation of $G_q\alpha$ requires rhodopsin, but none of the known downstream phototransduction components, suggesting that the signaling pathway triggering translocation occurs upstream of $G_q\alpha$. Finally, we show that *ninaC* mutants display a significantly reduced rate of $G_q\alpha$ transport from the cell body to the rhabdomere, suggesting that NINAC might function as a light-dependent plus-end motor involved in the transport of $G_q\alpha$.**

Key words: Light-dependent, Translocation, *Drosophila*, G-protein, NINAC, Myosin

## Introduction

An important property of photoreceptors is their ability to adjust their sensitivity in different levels of background illumination, allowing them to operate in a wide range of light intensities. One possible mechanism underlying light adaptation is the regulation of the number of signaling components available for activation. *Drosophila* photoreceptors contain two compartments: (1) the rhabdomere, the signaling compartment, which consists of ~60,000 tightly packed microvilli that house most of the phototransduction components; and (2) the cell body. Regulated translocation of signaling components between these subcellular regions might be an effective strategy for controlling the number of components available for signaling.

In the rhabdomeres, light stimulation of rhodopsin leads to the activation of a $G_q\alpha$ protein, which activates a phospholipase Cβ (PLC), eventually leading to the opening of the light-activated ion channels, transient receptor potential (TRP) and TRP-like (TRPL). Multiple components mediate deactivation of the light response, including the eye-specific protein kinase C (eye-PKC), calmodulin, Arrestin-2 and calmodulin-dependent protein kinase II (Byk et al., 1993; Hardie and Minke, 1993; Kahn and Matsumoto, 1997; Ranganathan et al., 1991; Scott et al., 1997; Smith et al., 1991).

Recent reports have suggested that translocation of TRPL channels in *Drosophila* photoreceptors and the G-protein transducin in vertebrate photoreceptors contributes to long-term light-adaptation (Bahner et al., 2002; Sokolov et al., 2002). In the dark, TRPL and transducin are localized to the rhabdomeres of photoreceptors and the outer segment of rods, respectively. In the light, both display massive translocation to the cell body and inner segment (Bahner et al., 2002; Brann and Cohen, 1987; Broekhuyse et al., 1987; Broekhuyse et al., 1985; Mangini and Pepperberg, 1988; McGinnis et al., 1992; Mendez et al., 2003; Philp et al., 1987; Sokolov et al., 2002; Whelan and McGinnis, 1988).

While our study was in progress, a report by Kosloff et al. (Kosloff et al., 2003) showed that *Drosophila* $G_q\alpha$ also translocates between the rhabdomere and cell body in a light-dependent manner. Here, we provide further characterization of $G_q\alpha$ translocation and provide the first evidence implicating the photoreceptor-specific class-III myosin NINAC in $G_q\alpha$ transport. We show that as much as 50% of $G_q\alpha$ translocates from the rhabdomere to the cell body within 5 minutes of illumination. When light intensity is increased over five orders of magnitude, translocation of $G_q\alpha$ out of the rhabdomere increases from 20% to 75%, consistent with a role in light adaptation. We then examine a range of genetic mutants to gain insight into the signaling pathway leading to $G_q\alpha$ translocation. We show that the translocation of $G_q\alpha$ requires the light activation of rhodopsin but does not require any of the known signaling components downstream of the G-protein. Finally, we show that, although *ninaC* mutants display normal translocation of $G_q\alpha$ from the rhabdomere to the cell body, they exhibit a significantly reduced rate of $G_q\alpha$ transport from the cell body to the rhabdomere. We suggest that NINAC might serve as a motor for the light-dependent redistribution of $G_q\alpha$ from the cell body to the rhabdomere.

## Materials and Methods

### Fly stocks

The following *Drosophila* strains were used: *cn bw* as wild type; *ninaE*$^{I17}$ (O'Tousa et al., 1985), *dgq*$^{1}$ (Scott et al., 1995), *norpA*$^{p41}$ (Bloomquist et al., 1988), *trpl*$^{302}$ (Niemeyer et al., 1996), *trp*$^{P343}$ (Scott et al., 1997), *inaC*$^{209}$ (Smith et al., 1991), *ninaC*$^{5}$ (Montell and Rubin, 1988), *arr2*$^{5}$ (Alloway and Dolph, 1999) and *shibere*$^{ts1}$ (Grigliatti et al., 1973). All flies were reared in the dark at 25°C except for *shibere*$^{ts1}$ flies, which were reared at 18°C. Mutant alleles were crossed into a *cn bw* background to eliminate retinal pigments that autofluoresce.

### Immunolocalization studies

Flies less than 5 days old were placed 9.3 cm from a white light source (Lambda LS 175W xenon arc lamp with 400-700 nm bandpass filter; Sutter Instruments, Novato, CA, or equivalent) for the indicated time. Intensities are indicated, as measured by an Extech 403125 digital light meter. All experiments were conducted at 24°C. After illumination, fly heads were immediately removed, placed on minutien pins (Fine Scientific Tools, Foster City, CA), and fixed in 3% paraformaldehyde in PBS for 1 hour on ice. Heads were then washed with PBS and incubated in 2.3 M sucrose overnight at 4°C. For dark-raised samples, identical fixing procedures were performed under dim red-light conditions. Heads were halved, oriented with the eyes facing upwards on an ultramicrotomy pin (Ted Pella, Redding, CA), and frozen in liquid nitrogen. 1-μm-thick sections were then cut using a Leica Ultracut UCT attached to an EM FCS cryo unit (Leica Microscopy and Scientific Instruments Group, Heerbrugg, Switzerland) at –81°C. Sections were incubated in blocking solution (1% bovine serum albumin and 0.1% saponin in PBS) for 30 minutes at room temperature and incubated with an antibody against $G_q\alpha$ (1:400) (Scott et al., 1995), TRPL (1:50) (Niemeyer et al., 1996), Arr2 (1:100) (Dolph et al., 1993) or rhodamine-conjugated phalloidin (1:500) (Molecular Probes, Eugene, OR) overnight at 4°C. Antibodies were received as a gift from C. S. Zuker (University of California, San Diego, CA). Rhodamine-conjugated goat-anti-rabbit secondary antibodies (Jackson ImmunoResearch, West Grove, PA) were used at 1:200 for 1 hour at room temperature in the dark. Slides were mounted with 90% glycerol and *p*-phenylenediamine (Sigma Aldrich, St Louis, MO). Light-exposed images in the figures were taken at the same exposure as dark-raised samples and brightened for illustration purposes to indicate $G_q\alpha$ localization in the cell body.

### Quantitative analyses

Tissue sections were visualized using an Olympus BX51 upright fluorescent microscope, and 10-bit images were acquired using an Olympus MagnaFire 2.0 digital camera S99806 for quantitation. Images were analysed using the 'Manual Tag' option provided by ImagePro Express (Media Cybergenetics, Silver Springs, MD). A fixed circular area that encompassed approximately 90-100% of the area of an individual rhabdomere was used to measure fluorescent signal intensity within the rhabdomeres and also to measure background intensity levels. Circles used to measure background intensity were placed just off the section to detect any autofluorescence coming from the mounting medium or slide. Because ommatidia are so densely packed in the retina and photoreceptor cell bodies occupy most of the section (Fig. 1), we were unable to measure background intensity levels from the tissue section itself. For quantification in the rhabdomeres, the circle was placed with its bottom edge at the base of the rhabdomere. Identical images acquired in phase-contrast mode were used as an aid to positioning the circle on fluorescent images. For each time point, 240-480 rhabdomeres were measured from eight to twelve sections, from three to eight flies. The R7 photoreceptor cell was not included in any of these analyses. After subtracting average background levels of signal, an average signal intensity and standard errors were calculated for each time point using Microsoft Excel (Microsoft) and Origin 6.0 (Microcal, Northampton, MA).

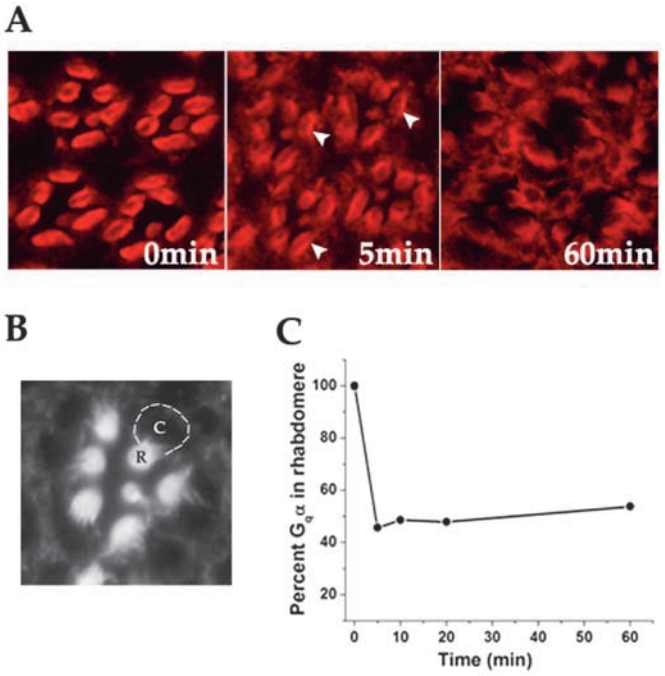



**Fig. 1.** Time-course of $G_q\alpha$ translocation from the rhabdomere to the cell body. (A) 1-μm-thick retinal cross-sections of wild-type flies that were dark-raised (0 minutes) or light exposed for 5 minutes or 60 minutes. Sections were stained with anti-$G_q\alpha$ antibodies. In dark-raised flies, $G_q\alpha$ displays complete rhabdomeric localization. Dark-raised flies exhibited $G_q\alpha$ immunoreactivity that was non-uniform across any one rhabdomere, displaying areas of higher concentration that appear to be randomly localized. By contrast, after 5 minutes of illumination, $G_q\alpha$ was progressively concentrated towards the base of the rhabdomere (arrowheads). After 60 minutes, we often observed localization of $G_q\alpha$ at the plasma membrane of the cell body. (B) A 1-μm-thick retinal cross-section of a single wild-type *Drosophila* ommatidium stained with phalloidin conjugated to rhodamine. The rhabdomere and cell body of a single photoreceptor cell are indicated by the letters R and C, respectively. For further clarification, the cell body is outlined. (C) Quantitation of $G_q\alpha$ in the rhabdomeres from cross-sections of dark-raised wild-type flies, exposed to light for 5 minutes, 10 minutes, 20 minutes or 60 minutes. Images used were taken at the same exposure time as dark-raised samples. Values are expressed as a percentage of $G_q\alpha$ signal measured in the rhabdomeres of dark-raised flies, in which $G_q\alpha$ protein is exclusively localized to the rhabdomeres. After 5 minutes of illumination at ~$50.7\times10^3$ lumens meter$^{-2}$, 54.4±0.63% of $G_q\alpha$ translocates out of the rhabdomere; no additional $G_q\alpha$ appears to be translocated out of the rhabdomere with longer light exposures. SEMs are indicated.

### Isolation of retinal membranes

For each light condition, 30 fly heads were collected on minutien pins, placed in Eppendorf tubes, and stored at –80°C. To prepare retinal extracts, fly heads were homogenized in buffer (20 mM Hepes, 30 mM NaCl, 5 mM EDTA, protease inhibitors, pH 7.5) and centrifuged (5000 rpm, 4°C, 1-2 minutes) to remove chiton (this process was repeated three times). Membranes were separated from cytosol by ultracentrifugation (55,000 rpm, 4°C, 30 minutes). Cytosolic fractions

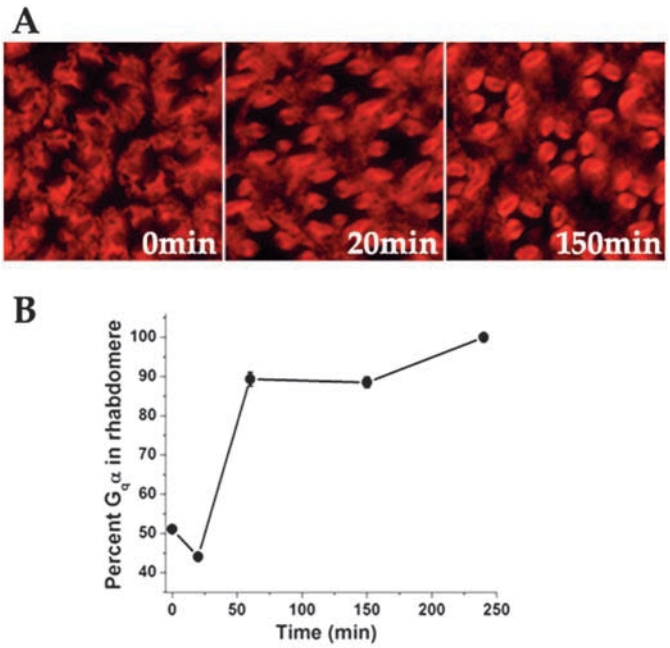



**Fig. 2.** Time course of $G_q\alpha$ translocation from the cell body to the rhabdomere. (A) Retinal cross-sections of dark-raised wild-type flies that were light exposed for 2.5 hours with a light intensity of ~$50.7\times10^3$ lumens meter$^{-2}$ and then incubated in the dark for 20 minutes or 150 minutes. Tissue sections were immunostained with an antibody against $G_q\alpha$. (B) Quantitation of $G_q\alpha$ in the rhabdomeres from cross-sections of wild-type flies that were light-exposed for 2.5 hours and then incubated in the dark for 20 minutes, 60 minutes, 150 minutes or 240 minutes. Values are expressed as a percentage of $G_q\alpha$ signal in the rhabdomeres of the corresponding dark-raised flies. In the wild type, 89.3±1.76% of $G_q\alpha$ is localized to the rhabdomeres within 60 minutes of dark incubation. SEMs are indicated.

were collected and concentrated using Centricon-10 columns (Millipore, Bedford, MA). Membrane fractions were resuspended in the above buffer solution, without EDTA. To quantify $G_q\alpha$ in fractions, 8-bit images were generated of immunoblots comparing membrane and cytosolic fractions. ImageQuant (Molecular Dynamics, Sunnyvale, CA) was used to quantify signal in protein bands. Background intensities were taken from the same blots, just near the band of interest.

## Results

### Light-dependent translocation of $G_q\alpha$ between the rhabdomere and the cell body

Previous quantitation and time-courses of $G_q\alpha$ translocation were based on its association with the membrane fraction of homogenates prepared from fly heads (Kosloff et al., 2003). Such biochemical assays test for a shift from membrane association to non-membrane association, regardless of subcellular localization. Because membrane-to-cytosol shifts are rather expected with Gα activation, a direct examination of the subcellular location of $G_q\alpha$ in different light conditions is required to gain insight into the phenomenon and underlying mechanisms. To address the subcellular translocation of $G_q\alpha$ between the rhabdomere and cell body, we performed immunostaining for $G_q\alpha$ on 1-μm-thick frozen sections from the eyes of dark-raised and light-exposed wild-type flies. Fig. 1B shows a wild-type retinal cross-section stained with rhodamine-conjugated phalloidin to highlight the two compartments of the photoreceptor (the actin-rich rhabdomere and the cell body). We found that, although $G_q\alpha$ is exclusively localized to the rhabdomeres of dark-raised flies, only 5 minutes of illumination by bright white light triggered the movement of ~50% of $G_q\alpha$ out of the rhabdomeres (Fig. 1). Levels of total $G_q\alpha$ protein remained constant, as determined by immunoblot analysis (data not shown). Under the given illumination conditions, 50% was the maximum quantity of $G_q\alpha$ mobilized, because 4 hours of illumination resulted in no additional translocation (data not shown). Because $G_q\alpha$ is required for phototransduction (Scott et al., 1995), we expect some $G_q\alpha$ to remain in the rhabdomeres to retain light-sensitivity. The time-course of $G_q\alpha$ translocation is similar to that of transducin in vertebrate rods (Sokolov et al., 2002).

Translocation of $G_q\alpha$ between the rhabdomere and cell body is reversible, because light-exposed flies returned to the dark displayed rhabdomeric localization (Fig. 2). To examine the time-course of translocation of $G_q\alpha$ from the cell body to the rhabdomere, we placed light-exposed flies in the dark for increasing time periods and quantified rhabdomeric $G_q\alpha$ immunostaining in tissue sections (Fig. 2). Movement of $G_q\alpha$ from the cell body to the rhabdomere was slower than translocation in the opposite direction. ~90% of $G_q\alpha$ returned to the rhabdomeres within 1 hour and full recovery required ~2.5 hours of dark incubation (Fig. 2).

If $G_q\alpha$ translocation is a mechanism for light adaptation then we would expect that higher background light intensities would lead to an increase in the quantity of $G_q\alpha$ transported out of the rhabdomeres and a decrease in the $G_q\alpha$ available for signaling. To test this, we examined $G_q\alpha$ translocation after 1 hour of exposure to different light intensities. Indeed, we found that the proportion of $G_q\alpha$ remaining in the rhabdomeres decreases with increasing intensities of light (Fig. 3A). 20-75% of $G_q\alpha$ translocated when exposed to light intensities increased over five orders of magnitude. These results demonstrate that light intensity regulates the quantity of $G_q\alpha$ protein available for signaling. Because a reduction in the amount of $G_q\alpha$ has been shown to decrease amplification (Hardie et al., 2002), $G_q\alpha$ translocation might be a mechanism underlying light adaptation.

### Rhodopsin is required for translocation of $G_q\alpha$ but PLC, TRP, TRPL and eye-PKC are not

The signaling pathway triggering translocation of $G_q\alpha$ is likely to include at least part of the phototransduction cascade. Kosloff et al. (Kosloff et al., 2003) showed that a constitutively activated form of the major rhodopsin Rh1 results in the persistence of non-membrane-bound $G_q\alpha$. In order to confirm that Rh1 is required for the subcellular translocation of $G_q\alpha$, we tested for $G_q\alpha$ translocation in a null mutant of Rh1, *ninaE*$^{I17}$. We performed immunostaining using an antibody against $G_q\alpha$ on tissue sections from the eyes of dark-raised and light-exposed mutant flies. Because *ninaE*$^{I17}$ mutants display retinal degeneration with age (Leonard et al., 1992; O'Tousa et al., 1989), we used young (<24-hour-old) flies whose photoreceptor structure was closer to normal. We found that $G_q\alpha$ is localized to the rhabdomeres of photoreceptors in both dark-raised and light-exposed *ninaE*$^{I17}$ flies (Fig. 4A). These

results demonstrate that Rh1 is required for $G_q\alpha$'s subcellular translocation.

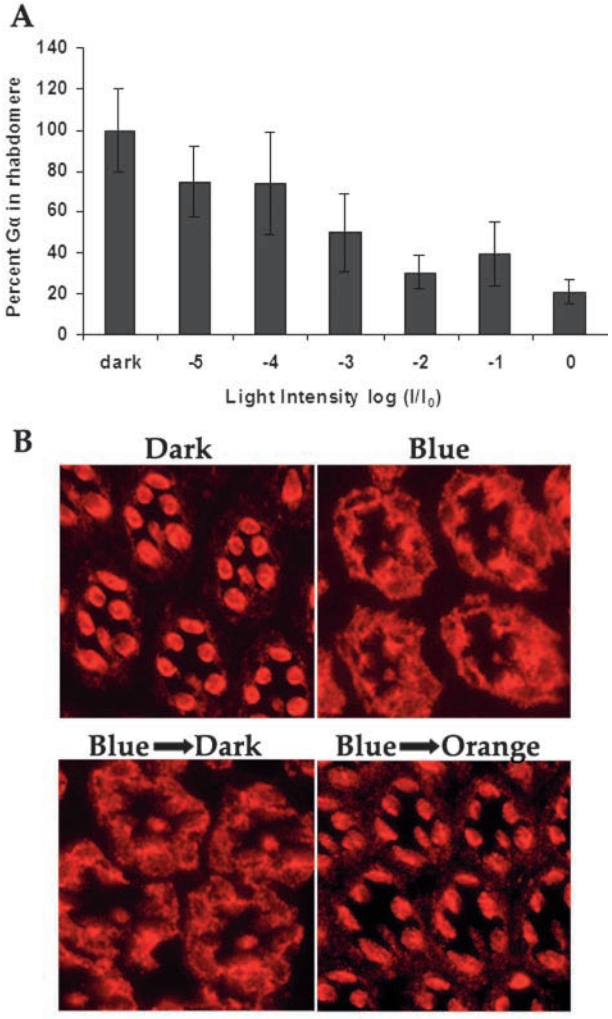



**Fig. 3.** (A) The quantity of $G_q\alpha$ translocated from the rhabdomere to the cell body is dependent on the intensity of illumination. Cross-sections of dark-raised wild-type flies were illuminated under the indicated light intensities for 1 hour. Sections were stained with anti-$G_q\alpha$ antibody and quantified. Percentages of $G_q\alpha$ remaining in the rhabdomeres after exposure to the indicated light intensities ($I$) were calculated, where initial light intensity ($I_0$)=~$70.0\times10^3$ lumens meter$^{-2}$. Exposure to light intensities with log($I/I_0$) of –5 to 0 resulted in translocation of ~20-75% $G_q\alpha$ out of the rhabdomeres. SEMs are indicated. (B) Photoisomerization of metarhodopsin to rhodopsin is required for $G_q\alpha$ transport from the cell body to the rhabdomere. Shown are retinal cross-sections immunostained for $G_q\alpha$. Dark-raised wild-type flies were exposed to: (1) blue (bandpass 470 nm > λ > 490 nm) light for 2 hours; (2) blue light for 2 hours followed by 2 hours of orange (long-pass λ > 580 nm) light; or (3) blue light for 2 hours followed by 2.5 hours of dark incubation. Dark-raised flies showed normal, rhabdomeric localization of $G_q\alpha$ whereas flies illuminated with blue light displayed translocation of $G_q\alpha$ into the cell body. Flies exposed to blue light followed by orange light displayed almost full recovery of $G_q\alpha$ to the rhabdomeres. Flies exposed to blue light followed by dark incubation showed no recovery of $G_q\alpha$ to the rhabdomeres. These results indicate that photoisomerization of metarhodopsin to rhodopsin is absolutely required for normal translocation of $G_q\alpha$ from the cell body to the rhabdomeres.

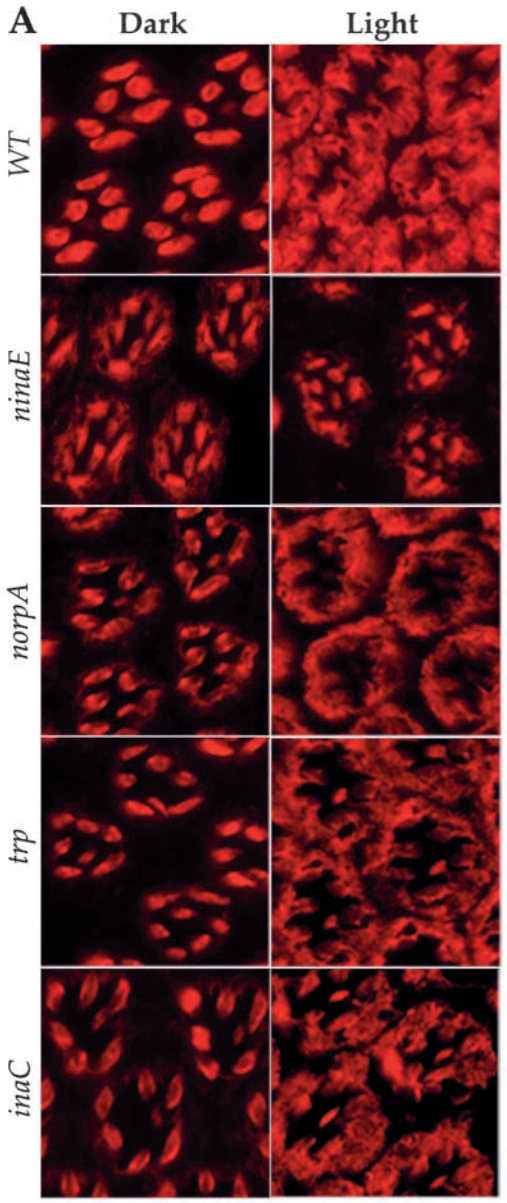
### Activation of rhodopsin to meta-rhodopsin is required for $G_q\alpha$ translocation

Unlike vertebrate rhodopsin, the major *Drosophila* rhodopsin Rh1 is maximally photoisomerized to the active meta-rhodopsin state with a wavelength ($\lambda_{max}$) of 480 nm (blue) light, whereas meta-rhodopsin is photoconverted back to rhodopsin with a $\lambda_{max}$ of 580 nm (orange) light (Minke, 1986). Because rhodopsin is required for $G_q\alpha$ translocation, it is possible that light activation either of rhodopsin to meta-rhodopsin or of metarhodopsin to rhodopsin triggers $G_q\alpha$ translocation. Because the white-light spectrum contains both blue and orange wavelengths, we used blue light alone (470 nm < λ < 490 nm) to test whether conversion of rhodopsin to meta-rhodopsin was sufficient to trigger translocation of $G_q\alpha$. In agreement with previous reports (Kosloff et al., 2003; Sokolov et al., 2002), there was no noticeable difference between $G_q\alpha$ translocation triggered by 480 nm light and that triggered by white light (Fig. 3B). These results show that photoisomerization of rhodopsin to meta-rhodopsin leads to $G_q\alpha$ translocation from the rhabdomere to the cell body.

The white light used in Fig. 2 also transmits orange light, so it was not clear whether dark incubation alone or photoisomerization of meta-rhodopsin to rhodopsin is required for $G_q\alpha$ translocation back into the rhabdomeres. To test this, we exposed wild-type flies to blue light (470 nm < λ < 490 nm) followed by orange light (λ > 570 nm) (Fig. 3B). Our results show that most, but not all, of $G_q\alpha$ was translocated back into the rhabdomere after 2 hours of orange-light exposure (Fig. 3B). Full recovery is not reached, probably because consistent activation of a small quantity of rhodopsin to meta-rhodopsin at 580 nm (Kirschfeld and Franceschini, 1977; Ostroy et al., 1974; Salcedo et al., 1999) prolongs the overall return of $G_q\alpha$ to the rhabdomere. Flies that were exposed to blue light followed by 2.5 hours of dark incubation displayed no translocation of $G_q\alpha$ back to the rhabdomere, suggesting that photoisomerization of meta-rhodopsin to rhodopsin is required for $G_q\alpha$ translocation from the cell body to the rhabdomere.

### PLC, TRP, TRPL and eye-PKC are not required for $G_q\alpha$ translocation

To determine which, if any, of the components downstream of $G_q\alpha$ in the phototransduction cascade are required for $G_q\alpha$ translocation to the cell body, we used null mutants for different components of the phototransduction cascade. Kosloff et al. (Kosloff et al., 2003) showed that, in PLC mutant (*norpA*) photoreceptors, $G_q\alpha$ loses its membrane association with illumination similar to the wild type, suggesting that PLC and activation of all downstream signaling components are not required for $G_q\alpha$ translocation. Surprisingly, however, $G_q\alpha$ translocation was blocked in a null mutant for the downstream TRP channel (Kosloff et al., 2003). Only $G_q\alpha$'s membrane association was tested in *norpA* mutants previously, and the requirement for TRP but not PLC is unclear because TRP acts downstream of PLC. To resolve these conflicting findings, we examined the subcellular localization of $G_q\alpha$ in retinal tissue sections from null mutants of: the effector PLC (*norpA*$^{P41}$), the two light-activated ion channels TRP (*trp*$^{343}$) and TRPL (*trpl*$^{302}$), and the eye-specific PKC required for deactivation of the cascade (*inaC*$^{209}$). We found that $G_q\alpha$ translocated from



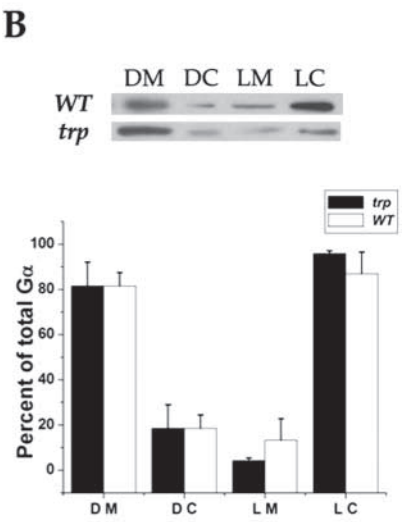




**Fig. 4.** $G_q\alpha$ translocation requires the light-activation of rhodopsin, but does not require the activation of the phototransduction components downstream of $G_q\alpha$. (A) Cross-sections of the wild type (WT) and null mutants in Rh1 (*ninaE*$^{I17}$), PLC (*norpA*$^{P41}$), TRP (*trp*$^{343}$) and PKC (*inaC*$^{109}$), all stained with anti-$G_q\alpha$ antibody. All flies were either dark raised or light exposed for 2 hours (~3000 lumens meter$^{-2}$). In *ninaE*$^{I17}$, $G_q\alpha$ is rhabdomeric in both dark and light conditions. A low level of $G_q\alpha$ signal was seen in cell bodies, consistent with previous reports showing that *ninaE* mutants display subrhabdomeric invaginations into the cell bodies (Leonard et al., 1992; O'Tousa et al., 1989). The remaining null mutants displayed rhabdomeric localization of $G_q\alpha$ in the dark and translocation of $G_q\alpha$ upon illumination. This suggests that rhodopsin is necessary for translocation of $G_q\alpha$, whereas PLC, TRP and PKC are not. (B) $G_q\alpha$ undergoes a light-dependent shift to membranes in *trp*$^{343}$ null mutants similar to WT. Dark-adapted WT and *trp*$^{343}$ flies were exposed to white light (~$50.7\times10^3$ lumens meter$^{-2}$) for 2.5 hours. Membrane and corresponding cytosolic fractions were isolated for immunoblot analysis. The bar graph shows the proportions of total $G_q\alpha$ present in the membrane and cytosolic fractions for dark-adapted and light-exposed flies. DC, dark-adapted cytosolic fraction; DM, dark-adapted membrane fraction; LC, light-exposed cytosolic fraction; LM, light-exposed membrane fraction. The *trp*$^{343}$ null mutants displayed a light-dependent shift of $G_q\alpha$ from the membrane-associated fraction to the cytosolic fraction that was not significantly different from the wild type. WT: DM, 81.5±6.0%; LM, 13.2±9.6%; *trp*: DM=81.5±10.5%, LM=4.2±1/3%. A representative immunoblot is shown above the graph. SEMs are indicated

the rhabdomere to the cell body upon light exposure in all of these mutants (Fig. 4A, Fig. 5A). Unlike TRPL translocation, which requires the presence of the TRP protein but not its function (Bahner et al., 2002), we find that translocation of $G_q\alpha$ does not require TRP in either capacity.

Our results for *trp* mutants contrast with those reported by Kosloff et al. (Kosloff et al., 2003), so we also compared the $G_q\alpha$'s light-dependent shift from membrane-association to cytosol in the wild type and in *trp* mutants. In wild-type flies, 82% of $G_q\alpha$ is present in dark-adapted membrane fractions, with only 13% present in light-exposed membrane fractions (Fig. 4B). In *trp* mutants, the shift of $G_q\alpha$ from the membrane to the cytosolic fraction was not significantly different from wild type (Fig. 4B; Student's *t*-test $P>0.05$), supporting our immunocytochemical data. Altogether, our results show that the signaling pathway leading to $G_q\alpha$ translocation begins with the light activation of rhodopsin to meta-rhodopsin but does not require the activation of phototransduction components downstream of $G_q\alpha$, including PLC, TRP, TRPL and eye-PKC.

## $G_q\alpha$ translocates independently of Arrestin and TRPL

Another component that acts upstream of $G_q\alpha$ and that might play a role in signaling $G_q\alpha$ translocation is Arrestin-2. In *Drosophila*, there are two eye-specific arrestin proteins, Arrestin-1 (Arr1) and Arrestin-2 (Arr2) (Hyde et al., 1990; LeVine et al., 1990; Smith et al., 1990; Yamada et al., 1990). Arr2 is five to seven times more abundant than Arr1 and *arr2* mutants display defects in deactivation (Dolph et al., 1993), consistent with its role of binding and deactivating meta-rhodopsin. The role of Arr1 is unclear, because *arr1* mutants do not exhibit any significant defects in their light responses except as an *arr1 arr2* double mutant (Dolph et al., 1993). Vertebrate visual arrestin has been reported to undergo light-dependent movement between the outer and inner segments of rods (Mendez et al., 2003), whereas Arr2 in *Drosophila* translocates between the rhabdomere and cell body of photoreceptors (Kiselev et al., 2000; Lee et al., 2003). Similar light-dependent translocation was also reported in crayfish (Terakita et al., 1998).

Because Arr2 acts upstream of the G-protein, it might also

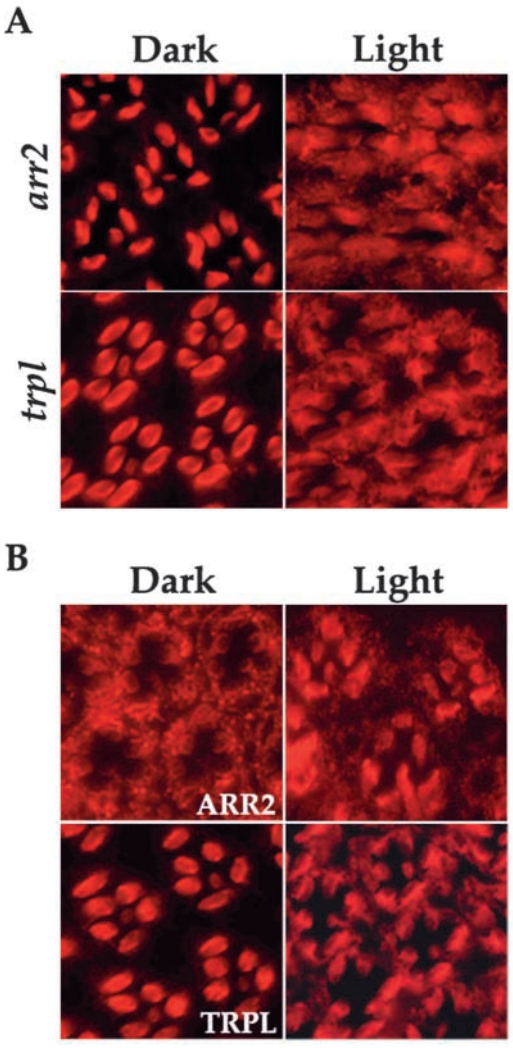



**Fig. 5.** (A) The translocation of $G_q\alpha$ is independent of TRPL and Arrestin-2. Cross-sections from *trpl*$^{302}$ and *arr2*$^{5}$ null mutants were stained with an antibody for $G_q\alpha$. All flies were either dark-raised or light exposed for 2 hours. $G_q\alpha$ displayed normal, light-dependent translocation in both the *trpl* and the *arr2* mutants, indicating their non-involvement in $G_q\alpha$'s translocation. (B) The translocation of TRPL and Arr2 is independent of $G_q\alpha$. Cross-sections from dark-raised and light-exposed *dgq*$^{1}$ mutants were immunostained for TRPL and Arr2. The *dgq*$^{1}$ mutant is a severe hypomorph that produces ~1% of the wild-type levels of $G_q\alpha$ (Scott et al., 1995). TRPL displays normal translocation, moving from the rhabdomere to the cell body upon illumination. Likewise, Arr2 displays normal light-dependent translocation from the cell body to the rhabdomere. These results indicate that the light-regulated translocation of both Arrestin2 and TRPL do not require $G_q\alpha$.

play a role in signaling the translocation of $G_q\alpha$. β-Arrestin has been reported to have multiple functions, including serving as a scaffold for two different signaling pathways (Luttrell et al., 1999; Zuker and Ranganathan, 1999). Because Arr2 and TRPL also undergo light-dependent translocation between the rhabdomere and cell body, albeit in different directions, (Bahner et al., 2002; Kiselev et al., 2000; Kosloff et al., 2003; Lee et al., 2003), we tested the possibility that $G_q\alpha$'s translocation might be dependent on Arr2 or TRPL. We found

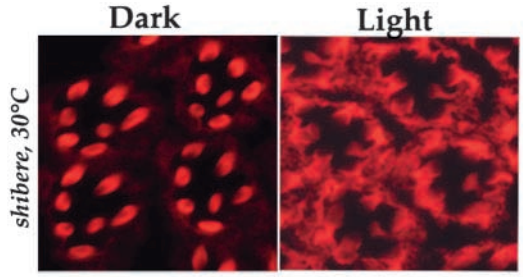



**Fig. 6.** The mechanism of $G_q\alpha$ translocation does not involve Shibere-mediated endocytosis. Dark-raised *shibere*$^{ts1}$ flies were first incubated at 30°C for 1-2 minutes in the dark to block endocytosis, as indicated by paralysis. Paralyzed *shibere*$^{ts1}$ flies continued to be incubated at 30°C but were either kept in the dark or transferred to the light for 1 hour. This incubation did not harm the flies because paralysis was reversible. Cross-sections were stained for $G_q\alpha$. When endocytosis is disrupted, $G_q\alpha$ still displays light-regulated translocation from the rhabdomere to the cell body.

that $G_q\alpha$ can translocate independently of TRPL and Arr2 (Fig. 5A), and that TRPL and Arr2 can translocate independently of $G_q\alpha$ (Fig. 5B). This is similar to visual arrestin and transducin in vertebrate rods (Mendez et al., 2003; Zhang et al., 2003). Although transport mechanisms for $G_q\alpha$, Arr2 and TRPL might or might not be shared, activation of components downstream of $G_q\alpha$ is not required for signaling translocation of $G_q\alpha$, Arr2 or TRPL.

## Translocation of $G_q\alpha$ is not regulated by shibere-mediated endocytosis

What is the molecular mechanism underlying $G_q\alpha$ translocation? Because vertebrate transducin displays light-dependent translocation to detergent-resistant lipid rafts (Nair et al., 2002), it is possible that $G_q\alpha$ translocation involves a membrane-associated type of transport, such as endocytosis. Previous studies have suggested that endocytosis mediates the movement of rhodopsin-arrestin complexes from the rhabdomeres to the cell body in the retinal degeneration mutant *rdgC* (Kiselev et al., 2000) and in *norpA* mutants (Alloway et al., 2000; Orem and Dolph, 2002). To test whether translocation of $G_q\alpha$ occurs through endocytosis, we used the temperature-sensitive *shibere* mutant *shi*$^{ts1}$. The *shibere* gene product is a homolog of the dynamin GTPase required in the 'pinching off' of vesicles during endocytosis (van der Bliek and Meyerowitz, 1991). At 25°C, *shi*$^{ts1}$ mutants are indistinguishable from wild type, whereas, at 29-30°C, *shi*$^{ts1}$ mutants display rapid paralysis as a result of disrupted endocytosis (Grigliatti et al., 1973; van der Bliek and Meyerowitz, 1991). To determine whether $G_q\alpha$ translocates from the rhabdomere to the cell body in photoreceptors via endocytosis, we examined the immunolocalization of $G_q\alpha$ in dark-raised and light-exposed *shi*$^{ts1}$ mutants incubated at the restrictive temperature. We found that $G_q\alpha$ was localized to the rhabdomeres in dark-raised *shi*$^{ts1}$ flies, whereas light exposure resulted in translocation of $G_q\alpha$ to the cell body similar to wild-type flies (Fig. 6). These results suggest that the molecular mechanism underlying $G_q\alpha$ translocation does not involve shibere-mediated endocytosis.

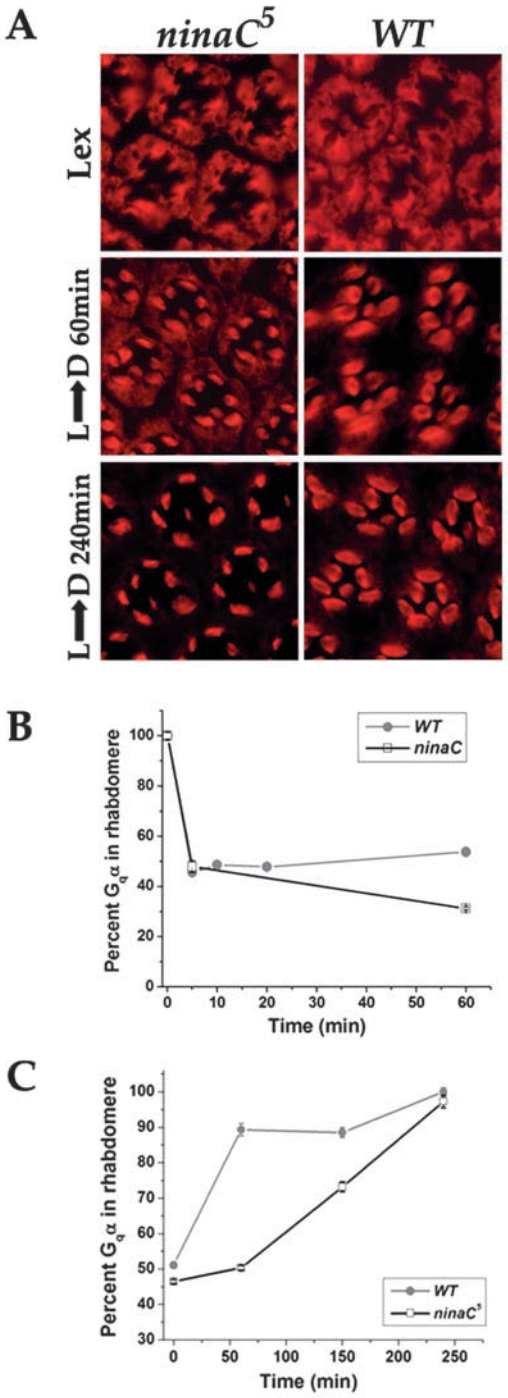



**Fig. 7.** The mechanism of $G_q\alpha$ translocation from the cell body to the rhabdomere involves the photoreceptor-specific myosin NINAC. (A) Cross-sections from wild-type (WT) and the null mutant *ninaC*$^5$ were immunostained for $G_q\alpha$. Flies were light-exposed for 2.5 hours (Lex) and then dark-incubated (L→D) for 0 minutes, 60 minutes or 240 minutes. Light-exposed WT and *ninaC*$^5$ flies displayed normal translocation of $G_q\alpha$ to the cell bodies. After 60 minutes of dark incubation, near-complete recovery of $G_q\alpha$ to the rhabdomeres was observed in the WT, whereas *ninaC*$^5$ mutants required up to 240 minutes of dark incubation for complete recovery of $G_q\alpha$ to the rhabdomeres. Total $G_q\alpha$ protein remained constant in light-exposed and dark-incubated samples as tested by immunoblot analysis (data not shown). (B) Quantitation of $G_q\alpha$ in the rhabdomeres from cross-sections of dark-raised wild-type (filled circles) and *ninaC*$^5$ (open squares) flies, exposed to light for 5 minutes or 60 minutes. Images used for quantitation were taken at the same exposure time as dark-raised samples. Values are expressed as a percentage of $G_q\alpha$ signal measured in the rhabdomeres of dark-raised flies. In both dark-raised WT and *ninaC*$^5$ flies, $G_q\alpha$ protein is exclusively localized to the rhabdomeres, whereas, after 5 minutes of illumination at ~$50.7\times10^3$ lumens meter$^{-2}$, the maximum amount (~50%) of $G_q\alpha$ translocates out of the rhabdomere. (C) Quantitation of $G_q\alpha$ in the rhabdomeres from cross-sections of WT and *ninaC*$^5$ flies that were light exposed for 2.5 hours and then incubated in the dark for 60 minutes, 150 minutes or 240 minutes. Values are expressed as a percentage of $G_q\alpha$ signal in the rhabdomeres of the corresponding dark-raised flies. The recovery of $G_q\alpha$ to the rhabdomeres was significantly slower in *ninaC*$^5$ mutants. In the WT flies, ~90% of $G_q\alpha$ is localized in the rhabdomeres within 60 minutes, whereas, in *ninaC*$^5$ mutants, only ~50% is localized in the rhabdomeres. After 60 minutes and 150 minutes of dark incubation, differences between WT and *ninaC*$^5$ were statistically significant (Student's *t*-test, $P<0.05$). Full recovery of $G_q\alpha$ to the rhabdomeres in *ninaC*$^5$ mutants was complete by 240 minutes.

## Photoreceptor-specific myosin NINAC is involved in the transport of $G_q\alpha$ from the cell body to the rhabdomere

Another possible mechanism of $G_q\alpha$ translocation is via a motor protein. In vertebrate photoreceptors, kinesin II and myosin VIIa have been suggested to function in the transport of opsin and arrestin from the inner segment to the outer segment (Marszalek et al., 2000; Williams, 2002). Because the rhabdomere is composed of microvilli, the major cytoskeletal component is actin. Therefore, a myosin is likely to be the molecular motor for $G_q\alpha$ translocation. NINAC is the only photoreceptor-specific myosin that has been identified thus far in *Drosophila* (Montell and Rubin, 1988). NINAC is a class-III myosin that is made up of an N-terminal protein-kinase domain, followed by a myosin head domain and two calmodulin-binding sites (Montell and Rubin, 1988; Porter et al., 1993). NINAC has been suggested to play a role in phototransduction (Porter et al., 1992; Wes et al., 1999a) as well as in linking the axial cytoskeleton to the microvillar membrane (Hicks et al., 1996; Hicks and Williams, 1992). Although mechanoenzymatic activity has not yet been demonstrated for *Drosophila* NINAC, a recent report by Komaba et al. (Komaba et al., 2003) showed that human myosin III functions as an actin-based motor protein with a translocating activity of 0.11 μm second$^{-1}$, as determined in an in vitro actin gliding assay. Because human myosin III acts as a plus-end motor (Komaba et al., 2003) and microvilli consist of bundled actin filaments polarized with their plus ends oriented away from the cell body (Arikawa et al., 1990; Mooseker et al., 1982), we expected that NINAC might transport $G_q\alpha$ from the cell body to the rhabdomere.

To test whether either of the NINAC isoforms (Montell and Rubin, 1988; Porter et al., 1992) is involved in the light-dependent translocation of $G_q\alpha$, we examined the time course of $G_q\alpha$ translocation in the *ninaC*$^5$ (also called *ninaC*$^{P235}$) null mutant. Because *ninaC* mutants have been shown to display light-dependent and age-dependent retinal degeneration, all experiments were performed using dark-raised, newly eclosed (<8 hours old) flies that display the minimum amount of degeneration (Porter et al., 1992). Our results show that the time course of $G_q\alpha$ transport from the rhabdomere to the cell

body was similar to the wild type (Fig. 7): maximal translocation was complete within 5 minutes. The rate of $G_q\alpha$ transport from the cell body to the rhabdomere, however, was significantly reduced (Fig. 7). The proportion of $G_q\alpha$ in the rhabdomeres after 2.5 hours of light exposure was similar between wild-type and *ninaC*$^5$ mutants: 51.1±0.9% and 46.5±0.5%, respectively. After 1 hour of dark incubation, we found that, in wild-type flies, 89.3±1.8% of $G_q\alpha$ is localized to the rhabdomeres, whereas only 50.3±0.6% of $G_q\alpha$ is localized to the rhabdomeres in *ninaC*$^5$ mutants. Almost full recovery of $G_q\alpha$ to the rhabdomeres was reached within 1 hour for the wild type, whereas full recovery of $G_q\alpha$ to the rhabdomeres of *ninaC*$^5$ mutants was observed after 4 hours of dark incubation. Our findings are consistent with a model in which NINAC functions as a plus-end-directed motor protein facilitating $G_q\alpha$ transport.

## Discussion

We report the light-dependent translocation of $G_q\alpha$ between the rhabdomeric compartment, where phototransduction occurs, and the cell body of *Drosophila* photoreceptors. In dark-raised flies, $G_q\alpha$ is localized to the rhabdomeres; illumination triggers massive translocation of $G_q\alpha$ to the cell body within 5 minutes. The quantity of $G_q\alpha$ transported is dependent on light intensity: increasing the light intensity over five orders of magnitude leads to the translocation of ~20-75% $G_q\alpha$. Similarly, ~90% of vertebrate transducin is reported to be shuttled from the outer segment to the inner segment of rod photoreceptors under saturating light intensities (Sokolov et al., 2002). Our results are consistent with $G_q\alpha$ translocation as a mechanism for light adaptation. Previous reports have shown that a reduction in the quantity of $G_q\alpha$ or the light-activated TRP channel in mutant photoreceptors results in a decrease in amplification (Hardie et al., 2002; Niemeyer et al., 1996; Scott and Zuker, 1998). Light-activated translocation of the TRPL channel has also been suggested to contribute to long-term light adaptation (Bahner et al., 2002). *trpl* mutants, however, do not display a complete loss of adaptation (Leung et al., 2000). We suggest that the translocation of $G_q\alpha$ and TRPL between the rhabdomere and cell body both contribute to light adaptation in *Drosophila* photoreceptors.

### Signaling pathway triggering translocation of $G_q\alpha$

Our genetic analyses show that $G_q\alpha$ translocation from the rhabdomere to the cell body requires the activation of rhodopsin to meta-rhodopsin, but not any of the known signaling components downstream of the G-protein, including PLC, TRP, TRPL, eye-PKC and Arr2. In agreement with our studies, Kosloff et al. (Kosloff et al., 2003) showed that a constitutively activated rhodopsin leads to the persistence of non-membrane-bound $G_q\alpha$ and that *norpA* mutants display a light-dependent shift of $G_q\alpha$ from the membrane-associated fraction to the soluble fraction of head homogenates. In contrast to our data, however, Kosloff et al. (Kosloff et al., 2003) report a requirement for the TRP channel in $G_q\alpha$ translocation. The reasons for these different results are unclear. Different experimental conditions, including light intensity, illumination time or fixation procedures following illumination, might have contributed to these conflicting results. We further demonstrate that the translocation of $G_q\alpha$ from the cell body to the rhabdomere requires the photoconversion of meta-rhodopsin to rhodopsin.

The signaling pathways following the light activation of rhodopsin and leading to $G_q\alpha$ translocation between the rhabdomere and cell body remain to be determined. Activation of $G_q\alpha$ might or might not be necessary for translocation. Pharmacological agents that block $G_q\alpha$ activation might be useful in determining whether activation of $G_q\alpha$ is required for translocation. One possibility is that, even without activating $G_q\alpha$, meta-rhodopsin might signal some other unidentified component leading to the mobilization of $G_q\alpha$. Another possibility is that activation of $G_q\alpha$ by rhodopsin is the trigger for translocation itself. Activated $G_q\alpha$ remains in the rhabdomeres to activate PLC and might then become a target for transport. Alternatively, activated $G_q\alpha$ might signal the translocation of inactive G-protein heterotrimers. In vertebrates and crayfish, $G\beta\gamma$ has been reported to translocate out of the outer segment and rhabdomeres, respectively, with illumination (McGinnis et al., 2002; Sokolov et al., 2002; Terakita et al., 1998; Zhang et al., 2003). The half-time to complete translocation of the transducin-$\alpha$ subunit from the outer to the inner segment was reported to be three times faster than that for the transducin-$\beta$ subunit (Sokolov et al., 2002), suggesting that the $\alpha$ and $\beta\gamma$ subunits translocate separately. In knockout mice lacking the transducin-$\alpha$ subunit, however, transducin-$\beta\gamma$ was unable to redistribute to the inner segment with light, suggesting that transducin translocates as a heterotrimer (Zhang et al., 2003). It has not yet been demonstrated whether the $G\beta\gamma$ subunit in *Drosophila* photoreceptors also moves out of the rhabdomere with illumination. If so, association with $G_q\alpha$ before, during and after transport will need to be assayed.

### Molecular mechanism underlying $G_q\alpha$ translocation

How is $G_q\alpha$ transported between the rhabdomere and cell body? We envisioned three potential mechanisms: (1) endocytosis of membrane-associated $G_q\alpha$; (2) transport by a myosin; and/or (3) diffusion of free $G_q\alpha$ from one compartment to the other. Because $G_q\alpha$ returns to the rhabdomeres significantly more slowly than it leaves, translocation in each direction might involve different components and/or mechanisms.

Our results show that translocation of $G_q\alpha$ is unaltered in the *shi*$^{ts1}$ mutant at the restrictive temperature, suggesting that shibere-mediated endocytosis is an unlikely mode of translocation. Endocytosis might instead eliminate unwanted components from the rhabdomere, because accumulated rhodopsin-arrestin complexes in *norpA* and *rdgC* mutants are removed by endocytosis (Alloway et al., 2000; Kiselev et al., 2000; Orem and Dolph, 2002).

Another possibility for transport of $G_q\alpha$ is a mechanism involving myosin(s). In this study, we show that the photoreceptor-specific myosin NINAC is required for a normal rate of $G_q\alpha$ transport from the cell body to the rhabdomere. We cannot, however, rule out the possibility that NINAC, which contains a protein-kinase domain and has been implicated as a signaling protein in phototransduction (Hofstee et al., 1996; Porter et al., 1995; Porter and Montell, 1993; Wes et al., 1999b), is involved in signaling $G_q\alpha$ translocation. *ninaC* null

mutants have also been shown to exhibit a loss of the axial cytoskeleton from rhabdomeres and undergo retinal degeneration (Hicks and Williams, 1992; Matsumoto et al., 1987), making it possible that slowed $G_q\alpha$ transport is due in part to rhabdomeric cytoskeletal degeneration. However, if slowed $G_q\alpha$ transport is indeed a secondary effect of retinal degeneration, we would expect the effect to be rather non-specific. Because our results show that only plus-end-directed translocation is affected in *ninaC* mutants, whereas minus-end-directed translocation is unaltered, we suggest that the slowed rate of $G_q\alpha$ transport is a direct effect of the loss of NINAC protein. Future analyses of additional *ninaC* mutant alleles will determine whether NINAC does indeed function as a motor protein in $G_q\alpha$ transport.

Interestingly, *ninaC* mutants do not display a complete blockage of $G_q\alpha$ translocation from the cell body to the rhabdomere. One possibility is that multiple myosins contribute to the transport of $G_q\alpha$. There are 12 other myosins in *Drosophila* (Tzolovsky et al., 2002). It will be important to determine whether any of these myosins are expressed in photoreceptors. Alternatively, in the absence of NINAC, $G_q\alpha$ might ultimately be translocated to the rhabdomeres by another molecular mechanism, such as diffusion. In wild type, NINAC might function in concert with diffusion to increase the rate of $G_q\alpha$ transport. Diffusion might contribute to translocation of $G_q\alpha$ in either direction. For example, when meta-rhodopsin activates the $G_q$ protein in phototransduction, the concentration of free $G_q\alpha$ is suddenly increased in the rhabdomeres, perhaps driving $G_q\alpha$ down its concentration gradient into the cell body. If diffusion drives $G_q\alpha$ back into the rhabdomeres with dark incubation then we expect that proteins binding to $G_q\alpha$ might play a role in regulating the concentration of free $G_q\alpha$. It will be important to determine whether, in *Drosophila* photoreceptors, $G_q\alpha\beta\gamma$ translocates as an inactive heterotrimer, $G_q\alpha$ and $G\beta\gamma$ translocate independently or $G\beta\gamma$ does not translocate. Other binding proteins of $G_q\alpha$ and $G\beta\gamma$ might also play essential roles in regulating the concentration of free G-protein subunits and their direction of translocation.

Subcellular localization of transduction proteins has proved to be crucial for signaling because mislocalization of components often results in the severe impairment of function. Dynamic regulation of protein localization might be an important strategy for controlling the quantity of transduction components available for signaling. In this way, cells can adjust their sensitivity and prevent overstimulation. The subcellular translocation of $G_q\alpha$ in *Drosophila* photoreceptors provides an attractive model for further investigation into the signaling pathway leading to translocation and the molecular mechanisms of transport.

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